

Applicant: David S. Lawrence  
Serial No.: 10/755,086  
Filed: January 9, 2004  
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Amendments to the Claims:

Please cancel claims 2, 5-48, 124-126, 132, and 135-136 without disclaimer or prejudice.

Please amend claims 61-63, 67, 71-72, 75, 77-81, 86, 123, 127, 129, and 134 as set forth below.

1. (Original) A method for identifying a chemical compound that inhibits a protein kinase, which comprises separately contacting the protein kinase with both the chemical compound and a fluorescently-labeled substrate for the protein kinase, and with the fluorescently-labeled substrate, under conditions suitable for phosphorylation of the fluorescently-labeled substrate by the protein kinase, and measuring fluorescence intensity, a smaller change in fluorescence intensity in the presence of both the chemical compound and the fluorescently-labeled substrate than in the presence of the fluorescently-labeled substrate indicating that the chemical compound inhibits the protein kinase; wherein the fluorescently-labeled substrate comprises a peptide and at least one fluorophore, wherein a fluorophore is attached to a serine, a threonine, or a tyrosine on at least one terminal end of the peptide, and wherein phosphorylation of the substrate by the protein kinase occurs at the terminal serine, the terminal threonine, or the terminal tyrosine to which the fluorophore is attached and produces at least a 20% change in fluorescence intensity.

2. (Canceled)

3. (Original) A method for identifying a chemical compound that inhibits a protein kinase in a living cell, which comprises comparing the fluorescence intensity when a fluorescently-labeled substrate for the protein kinase is introduced into a cell which has

not been contacted with the chemical compound, with the fluorescence intensity when the fluorescently-labeled substrate is introduced into a cell which has been contacted with the chemical compound, a smaller change in fluorescence intensity when the cell has been contacted with the chemical compound indicating that the compound inhibits the protein kinase in the living cell; wherein the fluorescently-labeled substrate comprises a peptide and at least one fluorophore, wherein a fluorophore is attached to a serine, a threonine, or a tyrosine on at least one terminal end of the peptide, and wherein phosphorylation of the substrate by the protein kinase occurs at the terminal serine, the terminal threonine, or the terminal tyrosine to which the fluorophore is attached and produces at least a 20% change in fluorescence intensity.

4. (Original) A method for determining if a protein kinase is active in a living cell, which comprises either introducing a fluorescently-labeled substrate for the protein kinase into the cell or contacting a lysate from the cell with the fluorescently-labeled substrate, and measuring fluorescence intensity, a change in fluorescence intensity indicating that the substrate has been phosphorylated by the protein kinase and that the protein kinase is active in the living cell; wherein the fluorescently-labeled substrate comprises a peptide and at least one fluorophore, wherein a fluorophore is attached to a serine, a threonine, or a tyrosine on at least one terminal end of the peptide, and wherein phosphorylation of the substrate by the protein kinase occurs at the terminal serine, the terminal threonine, or the terminal tyrosine to which the fluorophore is attached and produces at least a 20% change in fluorescence intensity.

5-48. (Canceled)

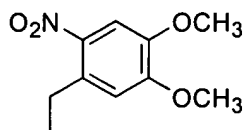
49. (Original) A substrate for a protein kinase, wherein the substrate comprises a peptide and at least one fluorophore, wherein a fluorophore is attached to a serine, a threonine, or a tyrosine on at least one terminal end of the peptide, and wherein phosphorylation by the protein kinase of the terminal serine, the terminal threonine, or the terminal tyrosine to which the fluorophore is attached produces at least a 20% change in fluorescence intensity.

50. (Original) The substrate of claim 49, wherein the substrate cannot be phosphorylated by a protein kinase until the substrate is activated.

51. (Original) The substrate of claim 50, wherein the substrate is activated by light.

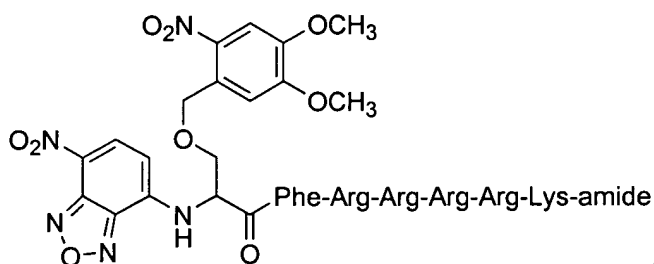
52. (Original) The substrate of claim 51, wherein the substrate comprises a serine, a threonine, or a tyrosine with a photolabile side chain that blocks transfer of a phosphoryl group from adenosine triphosphate to a hydroxyl moiety of the serine, the threonine, or the tyrosine.

53. (Original) The substrate of claim 52, wherein the photolabile side chain comprises the structure



54. (Original) The substrate of claim 52, wherein the substrate comprises a serine with a photolabile side chain that blocks phosphoryl transfer.

55. (Original) The substrate of claim 54, wherein the substrate has the structure

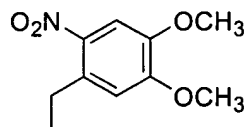


56. (Original) A substrate for a protein kinase, wherein the substrate comprises:  
a peptide comprising a serine, a threonine, or a tyrosine on a terminal end of the peptide;

at least one fluorophore, wherein a fluorophore is attached to the serine, the threonine, or the tyrosine on the terminal end of the peptide; and

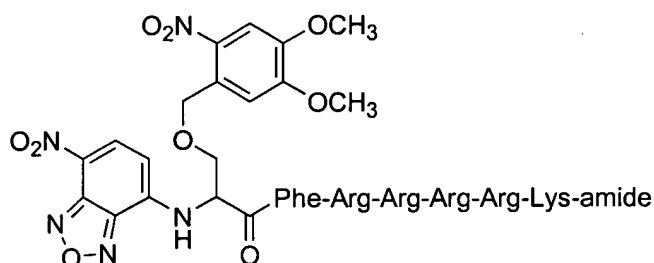
a photolabile side chain attached to the serine, the threonine, or the tyrosine on the terminal end of the peptide, wherein the photolabile side chain blocks transfer of a phosphoryl group from adenosine triphosphate to a hydroxyl moiety of the serine, the threonine, or the tyrosine so that the substrate cannot be phosphorylated by a protein kinase until the photolabile side chain is removed from the substrate.

57. (Original) The substrate of claim 56, wherein the photolabile side chain comprises the structure



58. (Original) The substrate of claim 56, wherein the substrate comprises a serine with a photolabile side chain that blocks phosphoryl transfer.

59. (Original) The substrate of claim 58, wherein the substrate has the structure



60. (Original) The substrate of claim 56, wherein after removal of the photolabile side chain, phosphorylation by a protein kinase of the terminal serine, the terminal threonine, or the terminal tyrosine to which the fluorophore is attached produces at least a 20% change in fluorescence intensity.

61. (Currently amended) The substrate of claim ~~49 or~~ 60, wherein the change in fluorescence intensity when the substrate is phosphorylated by the protein kinase is an increase in fluorescence intensity.

62. (Currently amended) The substrate of claim ~~49~~ 60, wherein the change in fluorescence intensity when the substrate is phosphorylated by the protein kinase is a decrease in fluorescence intensity.

63. (Currently amended) The substrate of claim ~~49~~ 60, wherein phosphorylation of the substrate by the protein kinase produces at least a 70% change in fluorescence intensity.

64. (Original) The substrate of claim 63, wherein phosphorylation of the substrate by the protein kinase produces at least a 100% change in fluorescence intensity.

65. (Original) The substrate of claim 64, wherein phosphorylation of the substrate by the protein kinase produces at least a 150% change in fluorescence intensity.

66. (Original) The substrate of claim 65, wherein phosphorylation of the substrate by the protein kinase produces at least a 250% change in fluorescence intensity.

67. (Currently amended) The substrate of claim ~~49~~ 56, wherein the substrate is specific for a protein kinase subtype.

68. (Original) The substrate of claim 67, wherein the substrate is specific for protein kinase C.

69. (Original) The substrate of claim 68, wherein the substrate is specific for isoforms  $\alpha$ ,  $\beta$ , and  $\gamma$  of protein kinase C.

70. (Original) The substrate of claim 67, wherein the substrate is specific for protein kinase A, protein kinase B, protein kinase D, protein kinase G, Ca<sup>+</sup>/calmodulin-dependent protein kinase, mitogen-activated protein kinase, protein kinase mos, protein kinase raf, protein tyrosine kinase, tyrosine kinase abl, tyrosine kinase src, tyrosine kinase yes, tyrosine kinase fps, tyrosine kinase met, cyclin-dependent protein kinase, or cdc2 kinase.

71. (Currently amended) The substrate of claim ~~49~~ or 56, wherein the substrate further comprises a carbohydrate, a lipid or a nucleic acid.

72. (Currently amended) The substrate of claim ~~49~~ or 56, wherein one fluorophore is attached to one terminal end of the peptide.

73. (Original) The substrate of claim 72, wherein the fluorophore is attached to the C-terminal end of the peptide.

74. (Original) The substrate of claim 72, wherein the fluorophore is attached to the N-terminal end of the peptide.

75. (Currently amended) The substrate of claim ~~49~~ or 56, wherein a fluorophore is attached to each terminal end of the peptide.

76. (Original) The substrate of claim 75, wherein fluorophores with distinct photophysical properties are attached to different terminal ends of the peptide.

77. (Currently amended) The substrate of claim ~~49~~ 56, wherein a first fluorophore is attached to a terminal end of the peptide and a second fluorophore, with photophysical properties distinct from the first fluorophore, is attached to any nonterminal site on the peptide.

78. (Currently amended) The substrate of claim ~~49~~ 56, wherein the fluorophore is a 7-nitrobenz-2-oxa-1,3-diazole derivative.

79. (Currently amended) The substrate of claim ~~49~~ 56, wherein the fluorophore is a fluorescein derivative.

80. (Currently amended) The substrate of claim ~~49~~ 56, wherein the fluorophore is selected from the group consisting of a dansyl derivative, an acridine derivative, an Alexa Fluor derivative, a BODIPY derivative, an Oregon Green derivative, a Rhodamine Green derivative, a Rhodamine Red-X derivative, a Texas Red derivative, a Cascade Blue derivative, a Cascade Yellow derivative, a Marina Blue derivative, a Pacific Blue derivative, an AMCA-X derivative, and a coumarin derivative.

81. (Currently amended) The substrate of claim ~~49~~ 56, wherein the fluorophore is attached to the peptide by a linker.

82. (Original) The substrate of claim 81, wherein the linker is a metal chelating linker.

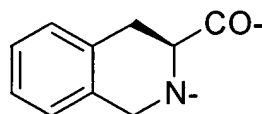
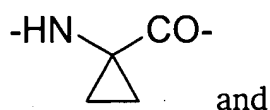
83. (Original) The substrate of claim 81, wherein the linker is selected from the group consisting of a carboxamide linker, an aminobenzoic acid linker, a sulfonamide



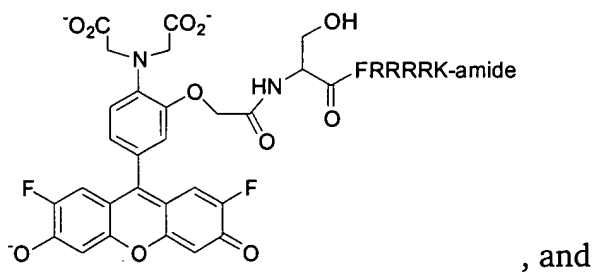
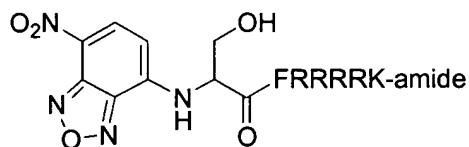
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linker, a urea linker, a thiourea linker, an ester linker, a thioester linker, an alkylamine linker, an arylamine linker, an ether linker, and a thioether linker.

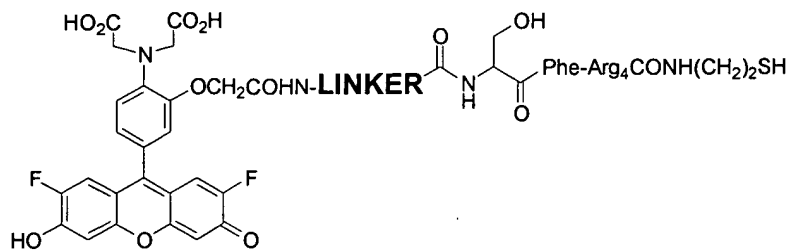
84. (Original) The substrate of claim 81, wherein the linker is selected from the group consisting of N-methyl glycine, L-proline, D-proline,



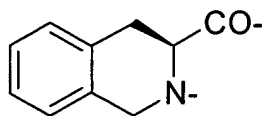
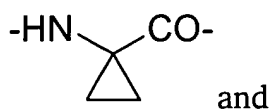
85. (Original) The substrate of claim 49, wherein the substrate is selected from the group consisting of:



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wherein F is phenylalanine, K is lysine, and R is arginine; and wherein the LINKER is selected from the group consisting of N-methyl glycine, L-proline, D-proline,

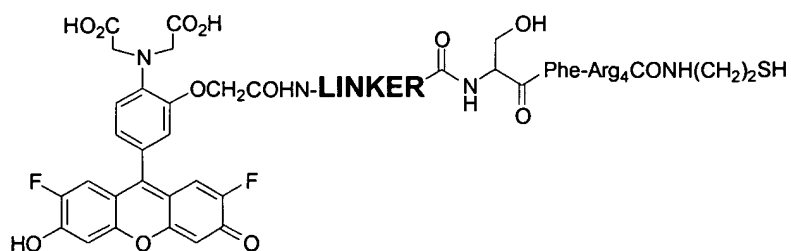


86. (Currently amended) A composition comprising the substrate of claim 49 or 56, and a carrier.

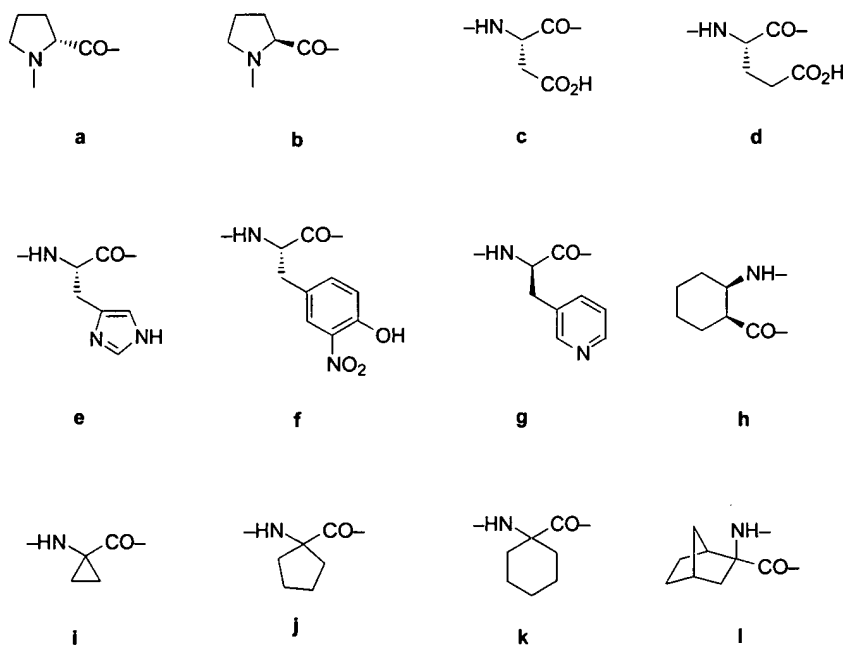
87. (Original) The composition of claim 86, wherein the composition is a pharmaceutical composition and the carrier is a pharmaceutically acceptable carrier.

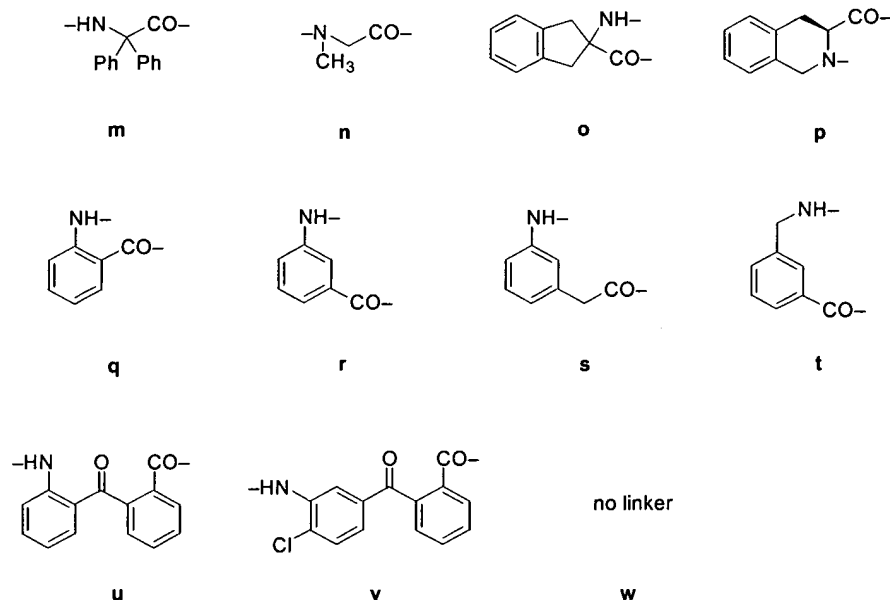
88. (Original) A chemical compound selected from the group of compounds set forth in Table 3.

89. (Original) A chemical compound having the structure:



wherein the LINKER is selected from the group consisting of the following:





90. (Original) A chemical compound having the structure:

fluorophore-LINKER-X-FRRRRK-amide (SEQ ID NO:3);

wherein F is phenylalanine; K is lysine; R is arginine; and X is serine, threonine, or tyrosine.

91. (Original) The chemical compound of claim 90, wherein the fluorophore is a 7-nitrobenz-2-oxa-1,3-diazole derivative.

92. (Original) The chemical compound of claim 90, wherein the fluorophore is a fluorescein derivative.

93. (Original) The chemical compound of claim 90, wherein the fluorophore is selected from the group consisting of a dansyl derivative, an acridine derivative, an Alexa Fluor derivative, a BODIPY derivative, an Oregon Green derivative, a Rhodamine Green

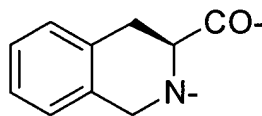
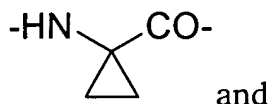
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derivative, a Rhodamine Red-X derivative, a Texas Red derivative, a Cascade Blue derivative, a Cascade Yellow derivative, a Marina Blue derivative, a Pacific Blue derivative, an AMCA-X derivative, and a coumarin derivative.

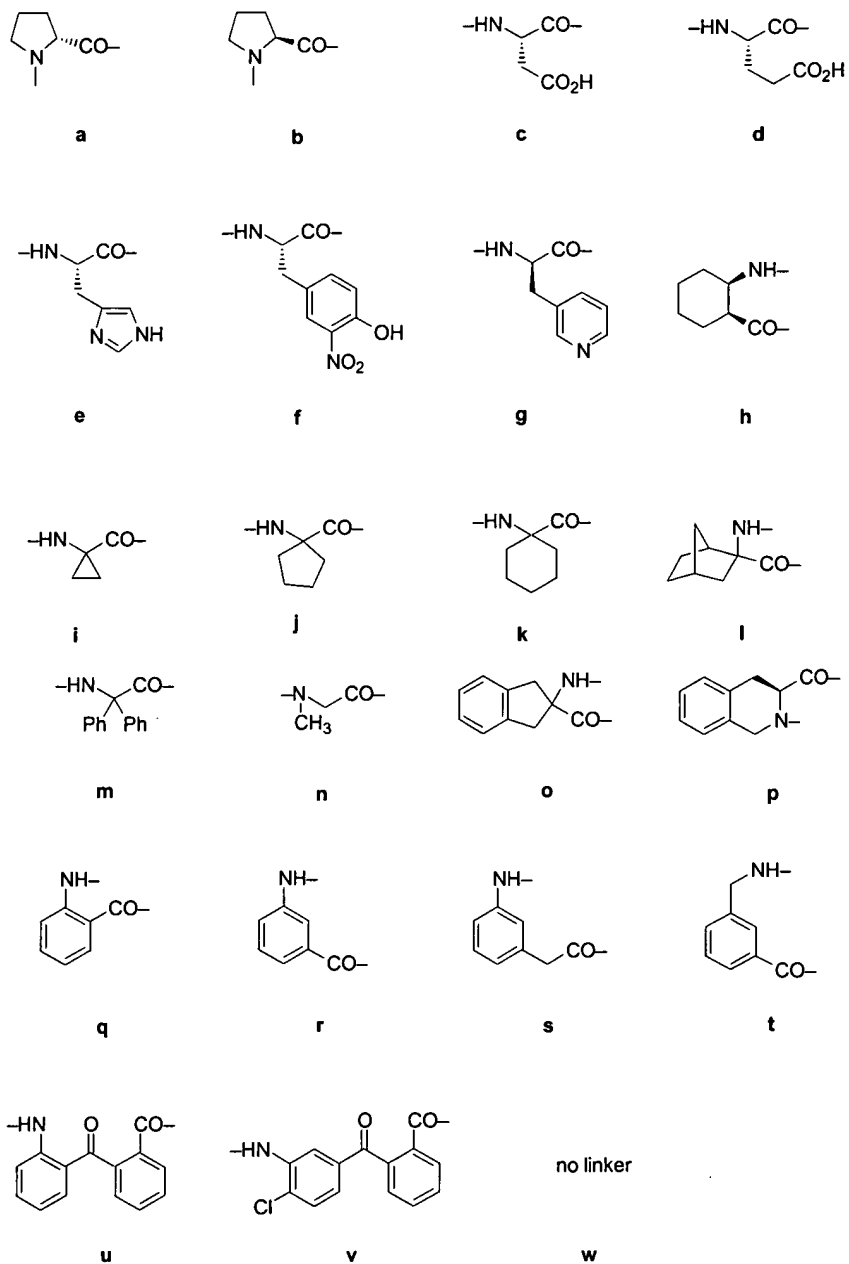
94. (Original) The chemical compound of claim 90, wherein the linker is a metal chelating linker.

95. (Original) The chemical compound of claim 90, wherein the linker is selected from the group consisting of a carboxamide linker, an aminobenzoic acid linker, a sulfonamide linker, a urea linker, a thiourea linker, an ester linker, a thioester linker, an alkylamine linker, an arylamine linker, an ether linker, and a thioether linker.

96. (Original) The chemical compound of claim 90, wherein the linker is selected from the group consisting of N-methyl glycine, L-proline, D-proline,



97. (Original) The chemical compound of claim 90, wherein the linker is selected from the group consisting of the following:



98. (Original) The chemical compound of claim 90, wherein the chemical compound is a substrate for a protein kinase.

99. (Original) The chemical compound of claim 98, wherein the chemical compound is specific for protein kinase C.

100. (Original) The chemical compound of claim 99, wherein the chemical compound is specific for isoforms  $\alpha$ ,  $\beta$ , and  $\gamma$  of protein kinase C.

101. (Original) The chemical compound of claim 98, the chemical compound is specific for protein kinase A, protein kinase B, protein kinase D, protein kinase G,  $\text{Ca}^{+}$ /calmodulin-dependent protein kinase, mitogen-activated protein kinase, protein kinase mos, protein kinase raf, protein tyrosine kinase, tyrosine kinase abl, tyrosine kinase src, tyrosine kinase yes, tyrosine kinase fps, tyrosine kinase met, cyclin-dependent protein kinase, or cdc2 kinase.

102. (Original) The chemical compound of claim 90, wherein the chemical compound further comprises a carbohydrate, a lipid or a nucleic acid.

103. (Original) A chemical compound comprising a peptide and at least one fluorophore, wherein a fluorophore is attached to a serine, a threonine, or a tyrosine on at least one terminal end of the peptide.

104. (Original) The chemical compound of claim 103, wherein the fluorophore is attached to the C-terminal end of the peptide.

105. (Original) The chemical compound of claim 103, wherein the fluorophore is attached to the N-terminal end of the peptide.

106. (Original) The chemical compound of claim 103, wherein a fluorophore is attached to each terminal end of the peptide.

107. (Original) The chemical compound of claim 106, wherein fluorophores with distinct photophysical properties are attached to different terminal ends of the peptide.

108. (Original) The chemical compound of claim 103, wherein a first fluorophore is attached to a terminal end of the peptide and a second fluorophore, with photophysical properties distinct from the first fluorophore, is attached to any nonterminal site on the peptide.

109. (Original) The chemical compound of claim 103, wherein the fluorophore is a 7-nitrobenz-2-oxa-1,3-diazole derivative.

110. (Original) The chemical compound of claim 103, wherein the fluorophore is a fluorescein derivative.

111. (Original) The chemical compound of claim 103, wherein the fluorophore is selected from the group consisting of a dansyl derivative, an acridine derivative, an Alexa Fluor derivative, a BODIPY derivative, an Oregon Green derivative, a Rhodamine Green derivative, a Rhodamine Red-X derivative, a Texas Red derivative, a Cascade Blue derivative, a Cascade Yellow derivative, a Marina Blue derivative, a Pacific Blue derivative, an AMCA-X derivative, and a coumarin derivative.

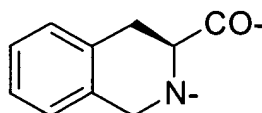
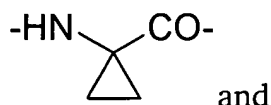


112. (Original) The chemical compound of claim 103, wherein the fluorophore is attached to the peptide by a linker.

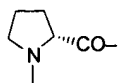
113. (Original) The chemical compound of claim 112, wherein the linker is a metal chelating linker.

114. (Original) The chemical compound of claim 112, wherein the linker is selected from the group consisting of a carboxamide linker, an aminobenzoic acid linker, a sulfonamide linker, a urea linker, a thiourea linker, an ester linker, a thioester linker, an alkylamine linker, an arylamine linker, an ether linker, and a thioether linker.

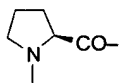
115. (Original) The chemical compound of claim 112, wherein the linker is selected from the group consisting of N-methyl glycine, L-proline, D-proline,



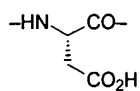
116. (Original) The chemical compound of claim 112, wherein the linker is selected from the group consisting of the following:



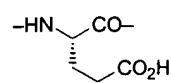
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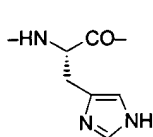
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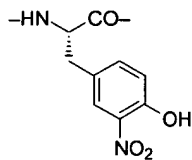
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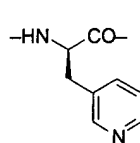
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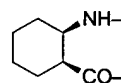
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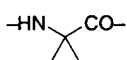
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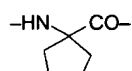
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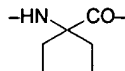
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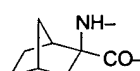
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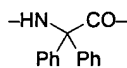
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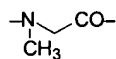
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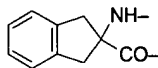
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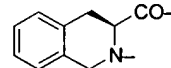
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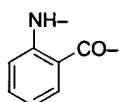
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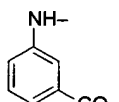
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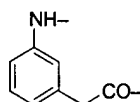
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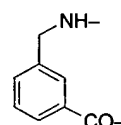
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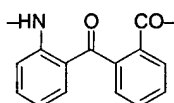
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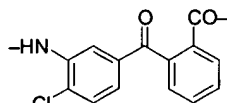
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no linker

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117. (Original) The chemical compound of claim 103, wherein the chemical compound is a substrate for a protein kinase.

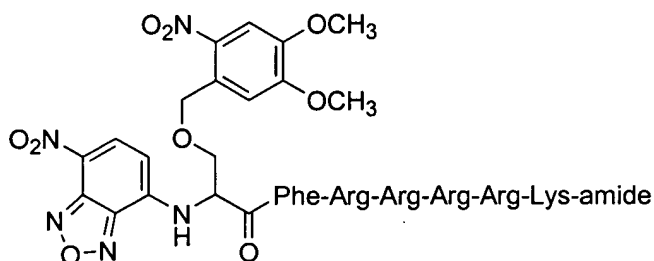
118. (Original) The chemical compound of claim 117, wherein the chemical compound is specific for protein kinase C.

119. (Original) The chemical compound of claim 118, wherein the chemical compound is specific for isoforms  $\alpha$ ,  $\beta$ , and  $\gamma$  of protein kinase C.

120. (Original) The chemical compound of claim 117, wherein the chemical compound is specific for protein kinase A, protein kinase B, protein kinase D, protein kinase G,  $\text{Ca}^{+}$ /calmodulin-dependent protein kinase, mitogen-activated protein kinase, protein kinase mos, protein kinase raf, protein tyrosine kinase, tyrosine kinase abl, tyrosine kinase src, tyrosine kinase yes, tyrosine kinase fps, tyrosine kinase met, cyclin-dependent protein kinase, or cdc2 kinase.

121. (Original) The chemical compound of claim 103, wherein the chemical compound further comprises a carbohydrate, a lipid or a nucleic acid.

122. (Original) A chemical compound having the structure



123. (Currently amended) A composition comprising a chemical compound of claim ~~88~~, 89, ~~90, 103 or 122~~, and a carrier.

124-126. (Canceled)

127. (Currently amended) The substrate of claim ~~49 or~~ 60, wherein a metal ion chelator induces the change in fluorescence intensity.

128. (Original) The substrate of claim 127, wherein the metal ion is a magnesium ion or a calcium ion.

129. (Currently amended) The chemical compound of claim ~~94 or 113~~, wherein a metal ion chelator induces a change in fluorescence intensity.

130. (Original) The chemical compound of claim 129, wherein the metal ion is a magnesium ion or a calcium ion.

131. (Original) The chemical compound of claim 129, wherein the change in fluorescence intensity is at least a 20% change in fluorescence intensity.

132. (Canceled)

133. (Original) The substrate of claim 81, wherein the linker comprises a turn to position the fluorophore in a location closer to the terminal serine, the terminal threonine or the terminal tyrosine than the location the fluorophore would occupy in the absence of a turn in the linker.

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134. (Currently amended) The chemical compound of claim 89, ~~90, or 112,~~ wherein the linker comprises a turn to position the fluorophore in a location closer to the terminal serine, the terminal threonine or the terminal tyrosine than the location the fluorophore would occupy in the absence of a turn in the linker.

135-136. (Canceled)